DATA EVALUATION RECORD

9/1/1999

PIRIMICARB

Study Type: 82-1a; 90-Day Oral Toxicity Study in Rats

Work Assignment No. 3-49B (MRID 44233103)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent

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DATA EVALUATION RECORD

STUDY TYPE: 90-Day subchronic toxicity [feeding] - rat
OPPTS Number: 870.3100 OPP Guideline Number: §82-1a

DP BARCODE: D236012 SUBMISSION CODE: S523927
P.C. CODE: 106101 TOX. CHEM. NO.: 359

TEST MATERIAL (PURITY): Pirimicarb (purity unspecified)

SYNONYMS: 5,6-Dimethyl-2-dimethylamino-4-dimethylcarboamoyl-oxy-pyridimidine; 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (IUPAC); 2-(dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate (CA); PP062

CITATION: Griffiths, D. and D.M. Conning (1995) First revision to ninety-day oral toxicity of PP062-albino rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory Project No. PR0106. May 24, 1995. MRID 44233103. Unpublished.

[Note: The original study was conducted in 1968 by Imperial Chemical Industries, now known as Zeneca Agrochemicals. The study was reformatted in this submission to include the required pages for an EPA submission under PR86-5. A full citation for the original study was not provided.]

SPONSOR: Zeneca Ag Products, 1800 Concord Pike, Wilmington, DE 19850

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44233103), pirimicarb (purity unspecified) was administered to albino Wistar rats (25 rats/sex/dose group) at 0, 250 or 750 ppm (males 0, 12.0 or 38.6 mg/kg/day, females 0, 15.3 or 47.2 mg/kg/day) in the diet or at 25 mg/kg/day by gavage for 90 days. There was no sham control group for the gavage animals. Following the 90-day treatment period, five rats in each group were maintained without treatment for a 28-day recovery period.

25 mg/kg/day - gavage group - Pirimicarb exerted a cholinergic effect which was observed at

1 week of dosing (the first time point measured) immediately (1 hour) after dosing in rats treated at 25 mg/kg/day by gavage. During the 90-day treatment period, plasma cholinesterase activity levels of gavage-dosed animals were 33-39% lower in males and 26-58% lower in females compared to corresponding control activity levels. During the 28-day recovery period, activity levels in both sexes approached concurrent control levels. No other treatment-related effects were observed in this group. The death or removal of 7 males and 5 females in the 25 mg/kg/day gavage-dosed group resulted from the trauma from repeated cannulation.

No treatment-related effects were observed in rats fed dietary concentrations of 250 or 750 ppm including differences in clinical signs, body weights, food consumption, hematology, erythrocyte or brain cholinesterase activity, absolute or relative organ weights, or gross or microscopic changes. Ophthalmoscopic, clinical blood chemistry, and urinalysis parameters were not measured. No neoplastic tissue was observed. **For the oral dosing (feeding) portion of the study, the NOAEL is 750 ppm (m - 38.6, f - 47.2 mg/kg/day), the highest dose tested; a LOAEL was not established. For the gavage dosing portion of the study, the LOAEL is 25 mg/kg/day, the only dose tested, based on plasma cholinesterase inhibition in both sexes; a NOAEL was not established.**

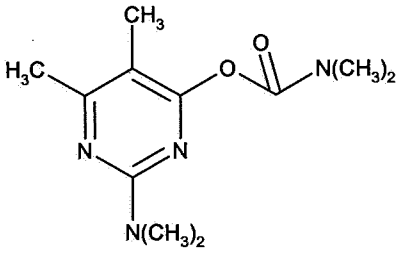
This 90-day subchronic toxicity study is classified **unacceptable(nonguideline) (82-1a)** because the test substance was inadequately characterized. The study may be upgraded and found to be acceptable(nonguideline) if complete characterization of the test substance is provided. This study is nonguideline by itself due to the study design with only 2 groups on dietary and 1 group of gavage rats. It does, however, provide useful information.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Pirimicarb
   Description: Colorless, odorless, crystalline liquid
   Lot/Batch #: Not reported
   Purity: Not reported
   Stability of compound: Not reported
   CAS #: 23103-98-2
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Rat
   Strain: Albino Wistar
   Age and weight at study initiation: Young adult (age not specified); males, 224-360 g; females, 170-252 g
   Source: Alderley Park, Cheshire, UK
   Housing: Five animals per cage in suspended Wilmslow-type Mobile Rat Units with galvanized wire mesh on three sides and the floor with a solid back
   Diet: Standard Pulverized Diet Chow, ad libitum
   Water: Not described, ad libitum
   Environmental conditions:
      Temperature: Not reported
      Humidity: Not reported
      Air Changes: Not reported
      Photoperiod: Not reported
   Acclimation period: Not reported

B. STUDY DESIGN:

1. In life dates - Not reported. The beginning of the 90-day treatment period was spread

over a period of 4 weeks.

2. Animal assignment

Rats (25/sex/group) randomly selected from a laboratory colony were assigned to the test groups in Table 1.

Table 1. Study design[a,b]

Test Group	Dose or concentration to Animal	Dose (mg/kg/day)		Animals Assigned	
				Male	Female
I	0	0		25	25
II	25 mg/kg (gavage)	25		25	25
		male	female		
III	0.025% (diet)	12.9[c]	15.3[c]	25	25
IV	0.075% (diet)	38.6[c]	47.2[c]	25	25

[a] Doses were selected based on results of preliminary studies that indicated that pirimicarb fed in the diet to rats at up to 45 mg/kg body weight was not toxic.

[b] Only five rats/sex/dose group were introduced to the dosing regime at any one time, so that terminal examinations could be conducted on 20 rats/sex/dose group at 90 days after study initiation. The remaining five rats/sex/group were maintained for a 28-day recovery period.

[c] Values taken from pages 123-4 (appendix A, Tables 22-3) of the study report. Doesn't include recovery group.

3. Treatment preparation and dosing

Dosing solution for rats treated by gavage (Group II) was prepared by ball-mixing pirimicarb in Dispersol OG for 24 hours. Diet for rats treated orally (Groups III and IV) was prepared by ball-mixing pirimicarb in maize oil for 18 hours, and incorporating the appropriate amount of mixture into standard pulverized stock diet. The frequency at which the gavage or feeding treatments were prepared was not reported. The concentration of pirimicarb in treated diet was stated to be confirmed using a modified reference method; no data were submitted. Data to confirm the homogeneity and stability of the test substance in the diet and/or dosing solution were not submitted.

Group II rats were administered dosing solution by stomach tube via a flexible cannula attached to a 5-mL syringe. Groups III and IV rats were fed dietary concentrations of pirimicarb. Group I rats were fed untreated standard pulverized stock diet.

4. Statistics

Body weights were analyzed for each sex using analysis of covariance on week 1 body weight. Weekly food consumption and food utilization during intervals of weeks 1-4, 5-8, and 1-13 were analyzed for each sex by analysis of variance. Hematology parameters (except for eosinophil counts and blood clotting measurements) were analyzed for each sex using analysis of variance on pre-experimental values. Eosinophil counts, kaolin-cephalin time, and pro-thrombin index were analyzed by analysis of variance at the time of sampling; data were analyzed for the combined sexes and the results examined to determine if any differences between the control and treated groups were consistent between sexes. The covariate adjustment was based on the separate sex pre-experimental group means. Erythrocyte and plasma cholinesterase for each sex were analyzed at the time of sampling by analysis of covariance on pre-experimental values. Brain cholinesterase was considered by analysis of variance for each sex. Organ weights were analyzed for each sex using analysis of variance and analysis of covariance. Least-squares means were calculated for each parameter and comparisons were made between each treatment and corresponding control group using a two-sided Student's t-test, based on the error mean square in the analysis. Significance was determined at the 5 and 1% levels.

C. METHODS:

1. Observations

Animals were examined daily during the study and any abnormalities were noted.

2. Body weight

Animals were weighed prior to treatment and once weekly during the treatment period.

3. Food consumption and compound intake

Food consumption (g) for each cage (5 rats/cage) was measured weekly during the study, and averaged on a per rat basis.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted during the study.

5. Blood

For hematology, blood was collected from 5 rats/sex/dose group prior to treatment and immediately prior to sacrifice at the end of the 90-day feeding period. It was not stated

whether the rats were fasted prior to blood collection. The CHECKED (X) hematology parameters were examined. Clinical blood chemistry parameters were not examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*	X	Mean corpusc. diameter
	Blood clotting measurements*	X	Reticulocyte counts
	(Clotting time)		
X	(Prothrombin time)		
	(Activated partial thromboplastin time)		
	(Kaolin/cephalin time)		
X			

* Required for subchronic toxicity studies.

6. Cholinesterase Activity

Cholinesterase activity was measured in plasma and erythrocyte fractions of blood from five rats per group. Blood samples were collected from each rat weekly for 5 weeks prior to study initiation, during study weeks 1 and 2, and at 2-week intervals to study termination. Because it had been established in preliminary studies that the cholinesterase effect disappeared by 4 hours after dosing, blood was collected from the gavaged rats 1 hour post-dosing. It was not specifically stated when blood was collected from the orally-dosed rats. Blood was also collected from the recovery group at 1 and 4 weeks following the cessation of dosing. Immediately after the final treatment, the brains from five other rats in each group were removed and brain cholinesterase activity was measured.

7. Urinalysis

Urinalysis was not conducted during the study.

8. Sacrifice and Pathology

At the end of 90 days or the recovery period, animals were killed with chloroform and subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	X	Brain* (cerebrum,
X	Salivary glands*	XX	Heart*		cerebellum, pons)
	Esophagus*		Bone marrow*	X	Periph.nerve*
X	Stomach*	X	Lymph nodes*		Spinal cord
X	Duodenum*	XX	Spleen*		Pituitary*
X	Jejunum*	X	Thymus*		Eyes (optic n.)*
X	Ileum*				
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
	Rectum*				
XX	Liver*	XX	Kidneys*	XX	
	Gall bladder*		Urinary bladder*		Adrenal gland*
X	Pancreas*	XX	Testes*		Lacrimal gland
			Epididymides	X	Mammary gland
			Prostate		Thyroids*
	RESPIRATORY	XX	Ovaries*		Parathyroids*
			Uterus*		
	Trachea*				OTHER
XX	Lung*				
	Pharynx				Bone*
	Larynx				Skeletal muscle*
					Skin*
					All gross lesions and masses*

* Required for subchronic toxicity studies.

II. RESULTS

A. Observations

1. Mortality - In the 25 mg/kg/day gavage treatment group, seven males and five females died or were removed from the experiment due to the trauma of repeated cannulation.

2. Clinical Signs - No treatment-related differences in clinical signs were observed in any test animal. All animals that survived the study duration were considered to be healthy throughout the treatment period.

B. Body weight and weight gain

No treatment-related differences in body weights or body weight gains were observed between rats in the treatment and control groups. Females in the 250 and 750 ppm dietary treatment groups and the 25 mg/kg/day gavage treatment group had lower body weights and body weight gains than the control females; the differences were attributed to very high individual body weights of two control females throughout the study. The differences in the female gavage group were also attributed to low individual body weights of two treated females, and high individual body weights of two control females. At the end of the 90-day treatment period, mean body weight gains for males were 137.6 g for the control group and 148.1-153.1 g for all treatment groups, and for females were 60.4 g for the control group, 51.0-53.3 g for the 250 and 750 ppm dietary treatment groups, and 41.0 g for the 25 mg/kg/day gavage treatment group. During the 28-day recovery period, no treatment-related differences in body weights or body weight gains were observed in any treatment group compared to the controls.

C. Food consumption and Compound Intake

No differences in food consumption were observed between the treated and control group rats.

Compound intake is presented in table 1.

D. Blood work - Hematology

No treatment-related differences in hematology parameters were observed between the treated and control group rats.

E. Cholinesterase Activity

a) Plasma cholinesterase - Rats treated at 25 mg/kg/day by gavage exhibited inhibition of plasma cholinesterase 1 hour following dosing compared to the controls. In preliminary studies, the inhibition was detected only within 4 hours of dosing. In the males, the activity was 39% lower at 2 weeks, 33% lower at weeks 4 and 8 and 38% lower at week 12 compared to the control activity levels (Table 2). In the females, the activity was 27 and 26% lower at weeks 1 and 2, 58% lower at week 4, 46% lower at week 8, and 36% lower at week 12 compared to control activity levels. Plasma cholinesterase activities in both sexes approached control levels during the 28-day

recovery period. Plasma cholinesterase activity levels of rats in the 250 and 750 ppm dietary treatment groups were similar to control activity levels.

Table 2. Plasma cholinesterase activity in male and female rats treated with pirimicarb for 90 days.[a]

Test Group	Treatment rate (mg/kg/day)	Plasma Cholinesterase Activity (μmol acetic acid/mL/min)						
		Week -1	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16[b]
Males								
I	0	0.57	0.53	0.51	0.61	0.66	0.66	0.76
II	25 (gavage)	0.56	d	0.31	0.41	0.44	0.41	0.69
III	25 (diet)	0.59	0.52	0.52	0.60	0.59	0.66	0.68
IV	75 (diet)	0.64	0.56	0.52	0.63	0.60	0.68	0.64
Females								
I	0	2.32	3.00	2.31	2.75	2.67	3.29	3.82
II	25 (gavage)	2.43	2.19	1.71	1.16	1.44	2.10	3.42[c]
III	25 (diet)	3.03	2.79	2.50	2.36	2.61	3.42	4.20
IV	75 (diet)	2.86	2.71	2.10	2.36	2.78	3.23	4.08

[a] Data were for 5 rats/sex/group for animals during the treatment and recovery periods, and were obtained from Table XIV, page 34 of the study report.
[b] Week 4 values during the 4-week recovery period.
[c] Mean of 4 females.
[d] Rats were not dosed prior to sampling.

b) Erythrocyte cholinesterase - No differences in erythrocyte cholinesterase activity were considered to be treatment-related in any test group.

c) Brain cholinesterase - No differences in brain cholinesterase activity were considered to be treatment-related in any test group.

F. Sacrifice and Pathology

1. Organ weight - No treatment-related differences in absolute or relative organ weights were observed between rats in the treatment and control groups.

2. Gross pathology - No gross abnormalities related to treatment were observed in treated rats.
3. Microscopic pathology

a) Non-neoplastic - No microscopic changes in rats from any treatment group were considered treatment-related.

b) Neoplastic - No neoplastic tissue was observed in rats from any test group.

III. DISCUSSION

A. Investigator's Conclusions

The study authors concluded that plasma cholinesterase activity was inhibited in rats treated with pirimicarb at 25 mg/kg/day by gavage 1 hour postdosing. No other effects were considered to be treatment-related in any test group.

B. Reviewer's Discussion

We agree with the study authors' conclusion that plasma cholinesterase inhibition in rats treated at 25 mg/kg/day by gavage was the only treatment-related effect observed in this study. Preliminary testing (not presented) showed that this effect lasted only 4 hours. Rats fed pirimicarb in the diet at amounts not quite double the amount administered by gavage did not exhibit plasma cholinesterase inhibition. The lack of plasma cholinesterase inhibition in rats fed dietary concentrations of pirimicarb confirms the causal relationship between the method of administration (oral versus gavage) and the manifestation of plasma cholinesterase inhibition. Specifically, orally-dosed rats had access to treated feed over an extended period of time (24 hours per feeding), whereas, gavage-dosed rats received the test substance in a single event. Carbamate compounds are relatively rapid reversible direct inhibitors of acetylcholinesterase that do not require metabolic activation. The temporary cholinergic effects observed in the rats treated by gavage are not unexpected, given that the dose was administered in a single event and did not require metabolic activation. During the 90-day treatment period, plasma cholinesterase activity levels of gavage-dosed animals were 33-39% lower in males, and 26-58% lower in females compared to corresponding control activity levels. During the 28-day recovery period, activity levels in both sexes approached concurrent control levels. Erythrocyte and brain cholinesterase activity were not inhibited in the gavage-dosed rats.

No other effects observed in the study were considered to be treatment-related. The death or removal of seven male and five female gavage-treated rats during the study was attributed to the physical trauma of repeated cannulation and not the treatment. No

treatment-related differences in clinical signs, body weights, food consumption, hematology, erythrocyte or brain cholinesterase activity, absolute or relative organ weights, or gross or microscopic changes were observed in any of the test groups. Ophthalmoscopic, clinical blood chemistry, and urinalysis parameters were not measured. No neoplastic tissue was observed.

For the test groups administered pirimicarb in the diet, the NOAEL is 750 ppm (m 38.6 and f 47.2 mg/kg/day), the highest concentration tested. For the test groups administered pirimicarb by gavage, the LOAEL is 25 mg/kg/day, the only concentration tested, based on plasma cholinesterase inhibition in both sexes. A NOAEL was not established.

IV. STUDY DEFICIENCIES

The original study was conducted in 1968 and contains scientific deficiencies and deviations that predate Subdivision F guideline requirements. The study was reformatted in this submission to include the required pages for an EPA submission under PR86-5.

The test substance was not adequately characterized. The purity, the manufacturer's lot and/or batch number, and the stability of the test substance were not reported. Data confirming the concentration of the test substance were not provided, although it was reported that the concentration was confirmed. Data confirming the homogeneity and stability of the test substance in the diet during the study were not submitted. In addition, clinical blood chemistry parameters were not measured in the test animals during the study.

The experimental design deviates from Subdivision F guidelines. A subchronic feeding study typically requires three dose levels to establish a dose response relationship and a NOAEL. The present study consists of two parts. The feeding portion of the study tested only two doses and did not establish a LOAEL. The gavage portion of the study tested only one dose. In addition, this study was initiated and ended over a 4 week period.

Despite these deficiencies and deviations, this study is considered scientifically valid in that it provides useful information pertaining to the relationship between cholinergic effects and the route of administration. The study may be considered acceptable(nonguideline) upon clarifying information regarding the deficiencies noted for complete test substance characterization.

SignOff Date:	9/1/99
DP Barcode:	D236012
HED DOC Number:	013708
Toxicology Branch:	RAB1